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Title: In vivo stability of WS1321 AASD strains carrying HIV peptide

Dates:

Purpose: Determine the number of bacteria recovered from the spleens of orally infected BALB/c mice. Compare the levels of each ^{bacterial} strain to that of the parent strain.

Strains: Constructed by Art Brantstrom are:

WS1321AASD, PAB102

WS1321AASD, PAB103

WS1321AASD, PAB102::gag

WS1321AASD, PAB103::gag

WS1321AASD, PAB102::rif

WS1321AASD, PAB103::rif

Also

WS1321 - plasmid - Sal. typhimurium strain

See to explain purpose
of construction of these 2
strains.

Note: A previous experiment conducted indicated by Day 6 after ^{oral} infection fewer numbers of strain WS1321, PAB103 were recovered from the spleens. ← Lab Notebook #3 (Mouse work)

Experiment: (Full procedure documented in) Lab Notebook #3 (Mouse work)

- strains were grown to an OD. of \approx 1.8 - 1.0
- strains to concentrate into DPBS (Some cultures resuspended in 0.35 ml. strains into 0.5 ml.)
- cultures were diluted & plated to determine ^{approx} bacterial fed.
- 40ul fed to mice using 200ul pipet.
- mice are to be sacrificed on Days 4, 7 & 11 post-feeding.
3 mice each strain each timept.

amt. given to mice: Between $3 - 4 \times 10^9$ in 40ul.

Exact #'s are in Book #3

* Spleens homogenized in 1ml HBSS 0.1ml original placenta duplicate

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PROJECT Salmonella Vaginale Canis

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Results: ave/spore = ave of $3 \times 1/0.5 \times 2$ ml dil.

Strain	Day	ave/spore	Day	ave/spore	Day	ave/spore
WS1321 #1	4	total ave/spore	7	total ave/spore	11	total ave/spore
# 2	131, 46	> 1697	98, 71	> 2097	111, 103	> 1690
# 3	70, 71		107, 97		65, 51	
WS1321 #1	44, 37	265	8, 9		63, 100	
PAB102 #2	14, 86	> 265	92, 135	> 1317	19, 17	> 1123
# 3	10, 66	1160	73, 78		74, 64	
WS1321 PAB103 #1	5, 4		35, 46	> 767	97, 57	
# 2	2, 9	> 70	59, 45	> 767	2, 3	> 1283
# 3	0, 0		26, 21		104, 122	
WS1321 PAB103 #1	0, 0		0, 0		3, 4	
# 2	0, 0	> 0	18, 19	> 507	0, 0	> 46
# 3	0, 0		54, 61		51	
WS1321 PAB103 #1	0, 0		0, 0		0, 0	
# 2	0, 0	> 0	0, 0	> 0	3, 0	> 10
# 3	0, 0		0, 0		0, 0	
WS1321 PAB103 #1	0, 0		0, 0		1, 2	
# 2	0, 0	> 0	0, 1	> 27	6, 9	> 63
# 3	0, 0		0, 0		4, 0	
WS1321 PAB103 #1	0, 0		0, 0		1, 0	
# 2	0, 0	> 0	0, 0	> 0	4, 4	> 53
# 3	0, 0		0, 0		3, 4	
<u>> 20 not detectable</u>						

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Cloning and Testing HIV genes under the control of the pgC promoter.

Art Blanckom constructed plasmid pAB104, which is plasmid pAB103 w/ the pgC promoter replacing the lac promoter.

* All data in Notebook #1 Digestions & Ligations

Using directed cloning techniques a 227 Kb fragment encoding Vif from HIV was cloned into pAB104

Bufler:

pAB104	5ul
B buffer	0ul
BamH1	1ul
dH2O	15ul
	20ul

pAB104: Vif	10ul
B buffer	2ul
BamH1	1ul
dH2O	7ul
	20ul

↓ 1 hr. 37°C

- add to each & continue at 37°C
for several hours.

Above 20ul

H buffer	3.2ul
Sal I	2ul
dH2O	14.8ul
	40ul

- + Each mix was run on a 1.2% low melt agarose gel. 1/2 mix on back side of the gel. With the gel was stained w/ EtBr after electrophoresis. The appropriate bands were sliced from the bottomed side
- Each band was Prep-A-Gene purified from through a Millipore column (a minicolumn) + resuspended in 25ul TE for ligation

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PROJECT Salmonella Vacuole Carriers

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Control → pAB104 cut, purified & ligated
 10X buffer 5 μl
 10mM ATP 4 μl
 pAB104 cut, purified 12.5 μl
 T4 Ligase 2 μl
 dH₂O 26.5 μl
 50 μl

pAB104 + vif
 10X buffer 5 μl
 10mM ATP 4 μl
 pAB104 12.5 μl
 Vif 2.5 μl
 T4 Ligase 2 μl
 dH₂O 15 μl
 50 μl

↓
 15°C, overnight in PCR machine
 15 min 12 min gel

- Ethanol prec. & electroporate into XG097AASO

Time (min)

colonies

Ligation Mix

pAB104 13.7

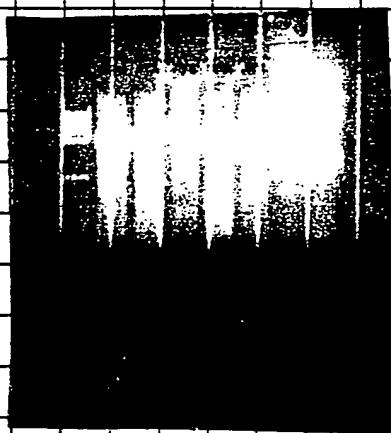
0

pAB104: vif 14.8

many.

5 positives were selected for digestion to look for inserts:

- Wilmad Miniprep - purify DNA
- Cut w/ BamH - SstI
- Run a gel.



all five appear to have inserts.

All five were prepared for Western Blot examination of expression levels. #10 were electroporated into JB501ThIO: AASO for

Placement into 1B21AASO

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PROJECT

Salmonella Vaccine Carriers

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Title: Macrophage Assay of pAgC construct

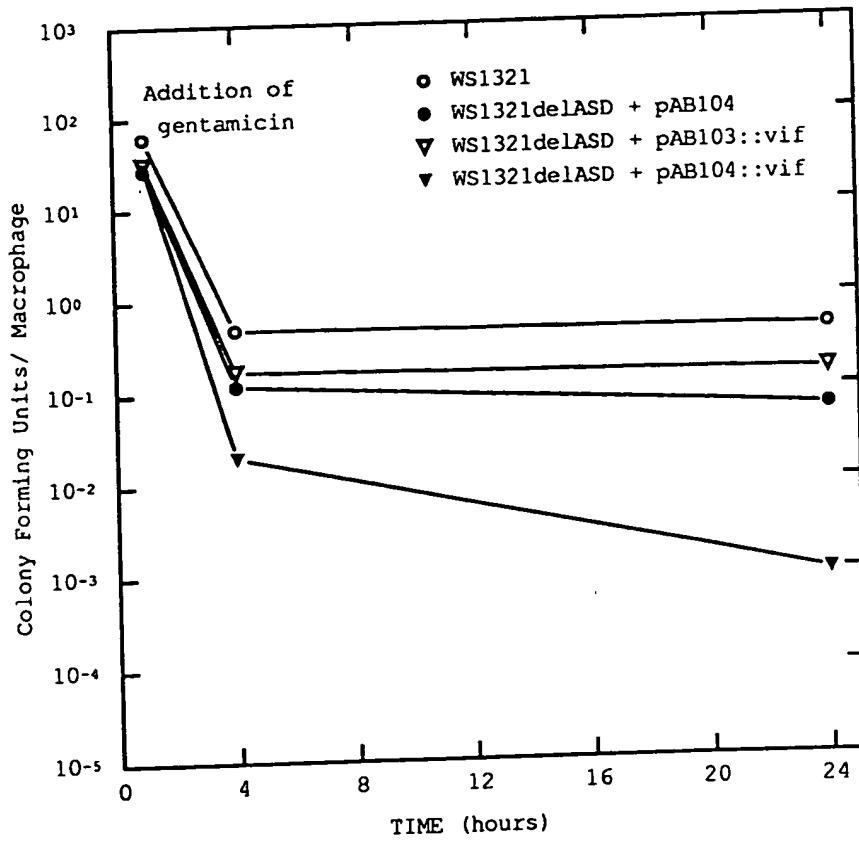
Purpose: Determine whether pAgC promotes increases expression in murine Macrophages

Notebook pag C #1 full experimental protocol.

Assay based on protocol of O'Brien et al.

CFU/Macrophage & Expression via Western blot were assayed.

Results:



Western in pag C notebook - Shows expression from pAgC (pAB104::vif only).

Slides of Macrophage infected in Petri dish - Notebook - pag C #1
Experiment Repeated

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Title: Growth of WS1321 Δ ASP + pAB104 in Minimal Media
 Supplemented w/ 0.1% Glucose

Notebook pag C #1

Repeated

Protocol:

- Single colony of each strain into 5ml of LB or MEM
- Grow 3-4 hrs.
- Concentrate & resuspend in dissociation buffer, water freeze & thaw
- Determine protein concentrations
- Run Western to compare LB & MEM expression of lacZ & pagC promoters.

Results: (Notebook pag C #1)

— 3/16 — EVP.

Bacterial Counts also determined

Lane # 10. Marker

< 9. WS1321

(LB) 8 pAB103::lacZ

7 pAB104

6 pAB104::lacZ

5 WS1321

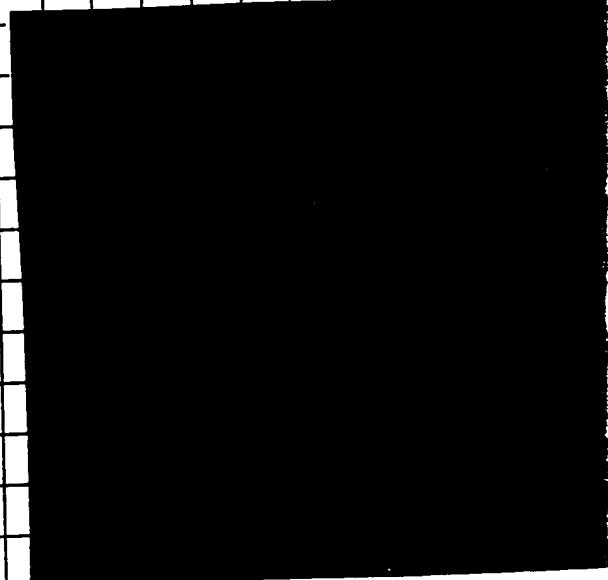
4 pAB103::lacZ

3 pAB104

2 pAB104::lacZ

1 2 3 4 5 6 7 8 9 10

1 2 3 4 5 6 7 8 9 10



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Title: Mouse Experiment page Controls
 Strains Tested: WS1321
 WS1321 A ASD+ pAB103::vif
 WS1321 A ASD+ pAB104
 WS1321 A ASD+ pAB104::vif

Purpose: Determine the number of bacterial colonies surviving mouse passage & expression of vif gene after passage.
 Mice sacrificed on days 3, 7 & 10

Amount of Bacteria Fed to Mice

40ml

WS1321	4.4×10^9
pAB103::vif	4.2×10^9
pAB104	6.9×10^9
pAB104::vif	6.4×10^9

Results: Experimental problem: Apparently mice were mixed by animal handlers. \leftarrow (Based on) Western blot analysis

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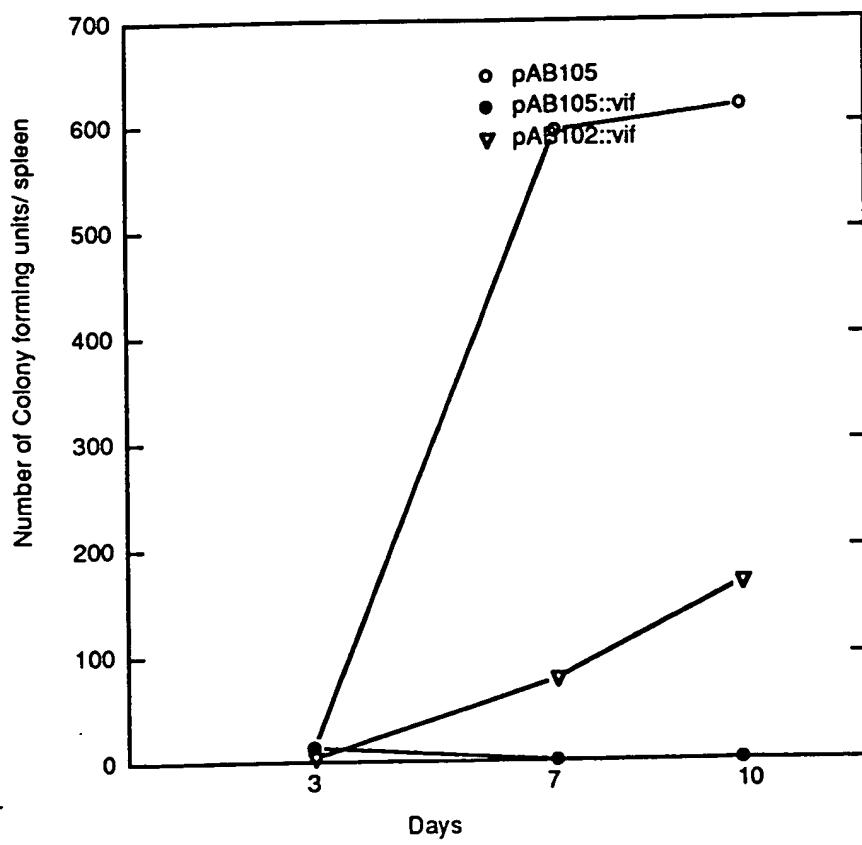
Title : Construction of WS1321AASD strains carrying pAB105 & pAB105::vif for mice feeding.

Purpose: pAB105 & pAB105::vif plasmids were constructed by^t
site directed mutagenesis. In this plasmid the asd gene is
from E.coli. We believe strains carrying plasmids
to this asd gene are healthier.

5/12 - Mice fed the following strains & amount
40ul

WS1321AASD + pAB105 3.6×10^9
WS1321AASD + pAB105::vif 2.72×10^9
WS1321AASD + pAB102::vif 6.4×10^9

Results: CFU/spleen + expression of vif from recovered colonies



Results of Western
Only pAB102::vif
showed significant
expression.

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Title: Construction of pAB105::ENV113

Purpose: Of the HIV genes we worked with only ENV has mapped restriction sites of a BALB/c mice.

After several attempts: → it was determined that the entire ENV gene isn't stable in our pGK plasmid. Various deletions resulted after each cloning attempt. Ended July

Will try smaller fragment which has been constructed by CT Random

End

~~Denote R. Segmire~~

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Title : Summary of Human Monocyte & S.typhi
Interaction Experiments. July

Note : This work has been in progress for some time.
This entry is a summary of the work completed
& a list of work needed to be done for a preblie

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Initial CTL assay

Purpose: To standardize non-radioactive CTL assay from Biomega. Testing various fetal calf serum lots.

Testing:

#1 FBS certified (Cow LDH \approx 470 units) Gibco
Lot # 40K0242

#2 Nyclone

5ml of #1 or #2

45ml of RPMI w/out Phenol red.

50 ml

- add 50ul to each well
- add 50ul of Control + LDH to 6 wells
- add 10ul of Cytosol solution
- add 50ul of Substrate Mix (12ml of Assay Buffer to bottle of Substrate Mix.) Cover plate w/ foil
- * incubate at RT for 30'
- Add 50ul of Stop Solution
- Remove large bubbles. Read Ab at 490.

Note - better control RPMI 1640 w/out serum.

Results: #1 best #2 cannot be used - completely red.
Aug 16 1994

Control Testing Maximum of Spontaneous Release of PSL5

#1 Group - RPMI

#2 Group - Media #2 - CTL Assay Media

Note: Cells are healthy. This is a must.

5×10^3 & 1×10^4 cells were tested for spontaneous immunoenzyme release.

RPMI w/out phenol red & Media #2 w/out phenol red. were used as control backgrounds.

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Results:

RDM 1
^{start Min.}
 5×10^3 0.479 0.138 $\times 3.5$
 1×10^4 0.748 0.158 $\times 4.7$

Media + 2
^{start Min.}
 0.537 0.153 $\times 3.5$
 1.083 0.150 $\times 7.22$

$$\text{RDI} = 0.074$$

$$\text{Media} = 0.162$$

$\downarrow 3\% \text{ FBS}$

$$\text{Gibco } 10\% = 0.326$$

Assay checked done it should work.

Test runs for CTL Proliferation & Cytokine
Assay in Shigella 2a, ENVIRO- \rightarrow experiment.

Genes inoculated by Coley Mallett.
ca

Above data contained in Shigella Cpp/Hela/Hep-2
Notebook.

Based on finds by Cut Brantum a pUC18 plasmid
encoding ENVIRO-A, present is stably maintained
in Shigella flexneri 2a without selection.

Cut Brantum has checked all strains used
for this study for Congo Red binding, Iba 1 expression
& Plasmid content.

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(Cloning of ENVI_B-A, plc ~...+ into pAB105)

Purpose: This ENVI_B-A fragment contains the relevant V3 loop, C-terminal epitope + major Ab epitope. Furthermore, it appears to be more stable in Plac8.

Outline of cloning procedure in rough notes: Pg C Notebook

Results: Appears to be cloned. Several weeks of problems with ligase!

Several Ab used for detection of expression

1. Human sera (Ab's)
2. Human sera (TD)
3. MAbs R9-2
4. V3 (HTB-V3-13) — only one that is good!

Mels Run

Doug / well protein
Comparing pAB102::ENVI_B-A, to pAB105::ENVI_B-A,
(plac) (pgc)

Repeated
Results:

- plac lysates from Art for 11-28-94
not enough protein loaded

- pgc expression is clearly higher than
plac.

Need to repeat w/ fresh Ab, but all looks good.
Set up for mouse testing.

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Stargate CM1 Response

59 mice total: 45th floor

Purpose: Test for CTL activity to EN1 epitope.

Mice sacrificed Sept. 6: Note: animals look ruffled.
- 2 S. flexneii 2a died.

Cells prepared for CTL, proliferation & cytokine assays.

Concept dropped

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PROJECT Shigella as a DNA Carrier

Previous background experiments appear in Notebooks I & II.

As of , to extend this concept we will use antibiotic treated BHK cells which have been infected w SC602(pCMV/p). The idea is that antibiotic treatment will kill the intracellular bacteria. The dead bacteria will release the pCMV/p plasmid into the cytoplasm for transcription & translation by the BHK cell.

This entry is based on a conversation with Jerry Soddy on [redacted]. This entry was [redacted].

- This entry was
Donna R. Simeone

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Lillian Van O Vugt
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Date

Esther Hartman

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